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FURTHER EVALUATION OF THE EFFECTS OF 7.5% NaCl/6% DEXTRAN-70 (HSD) ADMINISTRATION ON COAGULATION AND PLATELET AGGREGATION IN HEMORRHAGED AND EUVOLEMIC SWINE

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Division of Military Trauma Research

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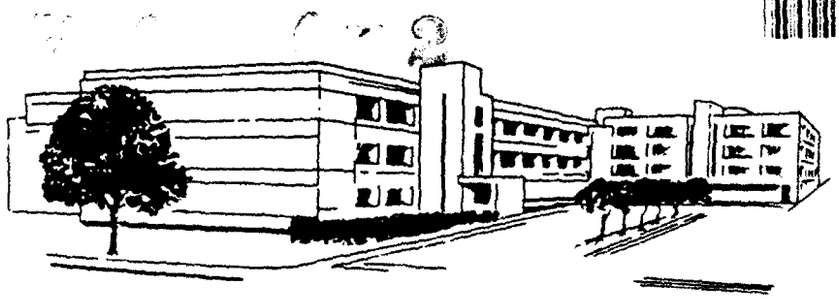

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Futher evaluation of the effects of 7.5% NaCl/6% Dextran-70 (HSD) administration on coagulation and platelet aggregation in hemorrhaged and euvolemic swine -- MA Dubick, AF Kilani, JJ Summary, JY Greene, and CE Wade

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HSD did not significantly affect PT, APTT, or platelet aggregation in either group of swine at any time measured. In other studies, HSD did not prolong bleeding times after 1 or 2 hr in euvolemic pigs. These data further support the premise that a single dose of HSD for the prehospital treatment of hemorrhagic hypotension will not adversely affect blood coagulation.

ABSTRACT

In previous studies, we determined that incubation of high concentrations of the 7.5% saline (HS) component of HSD with human blood, in vitro, significantly prolonged prothrombin time (PT) and reduced platelet aggregation. Considering the rapid plasma volume expansion which follows HSD infusion, the present study tested the hypothesis that any HS-induced effects on coagulation would have no clinical significance when HSD is infused for the treatment of hemorrhagic hypotension. Conscious, splenectomized pigs, either euvolemic (n=11) or bled 27 ml/kg over 60 min (n=9), were treated with the proposed therapeutic dose of 4 ml/kg HSD. Blood samples were withdrawn prior to, at the end of hemorrhage and 0.5, 1, 2, 3, 4, 24, 48, 72 and 168 hr following HSD infusion and PT, activated partial thromboplastin time (APTT) and platelet aggregation determined. HSD did not significantly affect PT, APTT, or platelet aggregation in either group of swine at any time measured. In other studies, HSD did not prolong bleeding times after 1 or 2 hr in euvolemic pigs. These data further support the premise that a single dose of HSD for the prehospital treatment of hemorrhagic hypotension will not adversely affect blood coagulation.

FURTHER EVALUATION OF THE EFFECTS OF 7.5% NaCl/6% DEXTRAN-70 (HSD) ADMINISTRATION ON COAGULATION AND PLATELET AGGREGATION IN HEMORRHAGED AND EUVOLEMIC SWINE -- M.A. Dubick, A.F. Kilani, J.J. Summary, J.Y. Greene and C.E. Wade

The past decade has seen a resurgence of research into the development of effective plasma volume expanders for the treatment of hemorrhagic hypotension. Of particular interest are hypertonic-hyperoncotic solutions that are effective in small volumes [1-3]. Over the past few years our laboratory [4,5] and others [6] have reported on the efficacy of infusions of 7.5% NaCl/6% Dextran-70 (HSD) as a small volume resuscitative solution in animal models of hemorrhagic hypotension. In addition, human field trials have reported that HSD improves survival in select groups of trauma patients [7,8].

However, before HSD can be acceptable for human use, concerns regarding its potentially adverse effects must be addressed. Of major concern are reports that dextrans can interfere with the blood clotting mechanism [9,10]. Although the incidence of clotting abnormalities are low considering the large number of doses of dextrans given over the past 50 years, clotting abnormalities have been most pronounced following infusion of large molecular weight (MW) dextrans (MW>130,000) or after the infusion of large doses (>1.5 g/kg body weight) of dextrans of average MW>60,000 [9,11,12]. In addition, recent studies have suggested that infusion of hypertonic saline (HS) alone can prolong clotting times and decrease platelet aggregation [13,14]. In our previous study examining the effects of high concentrations of HSD or its individual components, 7.5% saline (HS) and Dextran-70, in vitro, on coagulation and platelet aggregation, we observed significant HS-induced prolongation of blood coagulation [15].

These data, however, suggested that at the proposed therapeutic dose of 4 ml/kg, HSD, containing dextran of 70,000 average MW, would have minimal effects on hemostasis. To further examine whether these in vitro effects would be observed after IV infusion of HSD, plasma coagulation, platelet

aggregation and bleeding times were determined at times over a 7 day period in hemorrhaged and euvolemic swine infused with a single dose of 4 ml/kg.

MATERIALS AND METHODS

Animals and Experimental Protocol

Immature (25.2±1.4 kg) female Yorkshire pigs, (n=20) were randomly assigned to either the euvolemic (HSD) or the hemorrhage (hem+HSD) group. Animals were individually housed in a common indoor laboratory holding facility with a 12 hr light/dark cycle, were maintained at constant temperature and humidity, and were fed a commercial chow and water ad libitum. After a 1-3 week adaptation period to the laboratory environment, each pig was splenectomized and chronically instrumented with arterial catheters and an abdominal aortic sideport catheter to remove blood during hemorrhage [16]. A pyelostomy was also performed. All surgical procedures were performed under aseptic conditions, and the pigs were allowed to recover for 5 days before the start of each experiment. Specific aspects of the catheterization protocol and conditioning to the Pavlov sling have been previously detailed [17].

After an 18 hr fast, pigs were placed in the Pavlov sling and allowed to rest quietly. Two baseline blood samples (time 0) were collected. Immediately thereafter, animals in the hemorrhaged group were bled progressively at 27 ml/kg body weight over a 60 min period. The HSD solution (Pharmacia AB, Uppsala, Sweden) was then administered intravenously a 4 ml/kg body weight over a 1 min period. Blood samples (5 ml) were taken at the end of the hemorrhage or mock-hemorrhage period and at 0.5, 1, 2, 3, 4, 24, 48, 72, and 168 hr after HSD infusion.

Physiological and Biochemical Measurements

Mean arterial pressure (MAP) and heart rate were determined from the pulse pressure tracing recorded at baseline, during hemorrhage, and over the first 4 hr after HSD infusion.

Biochemical Measurement

Total carbohydrate concentrations in serum were determined by the anthrone reaction after precipitation of serum protein with 10% trichloroacetic acid (TCA) [18]. Plasma glucose was determined by an automated glucose-hexokinase enzymatic method performed by the Analytical Chemistry Branch, Letterman Army Institute of Research. Serum dextran concentrations were then calculated by subtracting the concentrations of glucose from the concentrations of total carbohydrate [19].

Coagulation and Platelet Aggregation Assays

Plasma coagulation was measured by prothrombin time (PT) and activated partial thromboplastin time (APTT), as well as platelet aggregation. Tests for PT and APTT were run in duplicate. All plasma controls used reagents of the same lot number.

Prothrombin time was assayed by incubating a 0.1 mL aliquot of the citrated plasma and thromboplastin (0.2 mL; Organon Teknika Corp., Durham, NC). The timer was started at the same time the reagent was added and clot formation was measured using a fibrometer (Helena Laboratories, Beaumont, TX).

The APPT was determined by incubating a 0.1 mL aliquot of plasma with 0.1 mL PTT activator (General Diagnostics, Morris Plains, NJ) and platelet factor 3 reagent for 5 min at 37°C; 0.2 mL $CaCl_2$ was added and the clotting time measured in the fibrometer.

Platelet aggregation was measured according to the standard platelet-rich plasma (PRP) method of Born [20]. Protein-rich plasma was processed from citrated whole blood by centrifugation at 150g for 10 min. Platelet concentrations were adjusted to 300,000/ L using autologous platelet-poor plasma. The PRP and an appropriate dilution of test solution in a final volume of 0.45 mL were added to a siliconized cuvette with a Teflon-coated stir bar. An agonist was added (0.5 mL), and the percentage of platelet aggregation was measured in the optical channel of a Whole Blood Aggregometer (Model 540, Chrono-Log Corp., Havertown, PA). Aggregation with

each agonist was measured in duplicate. Preliminary studies of platelet aggregation in pigs using the standard aggregation agonists epinephrine (10 mol/L), ADP (0.2 mol/L), collagen (0.2 mg/mL), and ristocetin (1.5 mg/mL; Bio/Data Corp., Hatboro, PA) revealed that pig platelet aggregation responded similarly to human platelets only with ADP as agonist. Therefore, ADP was used in subsequent studies to evaluate the effects of HSD on platelet aggregation.

Sedimentation Rates

Sedimentation rates of erythrocytes before and at times after HSD infusion were determined in Wintrobe tubes (Scientific Products, McGaw Park, IL) according to standard clinical laboratory procedures. Data were recorded as mm fall of erythrocytes/hr and were corrected for changes in hematocrit from baseline values.

Bleeding Times Study

Bleeding time determinations were performed using the ear incision technique described by Bowie et al.[21]. Due to technical limitations of this technique, only three time points were available from 4 euvolemic pigs infused with HSD. Bleeding times were determined at baseline (time 0), 1 hr, and 2 hr after HSD infusion. The effects of HSD on bleeding time were assessed in euvolemic rather than hemorrhaged pigs because vasoconstriction of the blood vessels in the pig ear could complicate the interpretation of the data from the hemorrhaged animals.

Statistical Analysis

Where appropriate, data were analyzed by Student's test or two-way analysis of variance (ANOVA), adjusted for repeated measures, with $P<0.05$ considered significant. The Newman-Keuls method of multiple comparison was employed to determine significant differences between the means [22]. In studies of dextran clearance from serum, an amended BMDP nonlinear regression statistical program was utilized for data analysis and for estimating half-life ($t_{1/2}$) [23].

RESULTS

In the present study, a 27 ml/kg hemorrhage resulted in a 42% decrease in mean arterial pressure (MAP) that was restored to baseline values following HSD infusion (Table I). Hemorrhage and the subsequent HSD infusion did not significantly affect heart rate in these animals, and in the euvolemic animals, HSD affected neither MAP nor heart rate (Table I).

Serum dextran concentrations were significantly higher in hemorrhaged pigs infused with HSD compared with their euvolemic controls (Fig. 1). Nevertheless, serum dextran concentrations decreased in both groups at a half-life of approximately 12 hr (12.3 ± 0.4 and 12.2 ± 1.0 hr in HSD and hem+HSD groups, respectively).

Serum Na concentrations peaked at 161 ± 3 and 156 ± 2 mEq/L in the HSD and hem+HSD groups, respectively, 2 hr after HSD infusion, corresponding to approximately a 10 mEq/L increase in each group (Table II). Na concentrations decreased thereafter until they had essentially returned to baseline values by 24 hr after HSD infusion. Cl concentrations increased 7 to 8 mEq/L following HSD infusion and returned to baseline values by 24 hr (Table II). K concentrations were not significantly affected by HSD infusion (Table II). Serum electrolyte concentrations were at baseline levels at 48, 72 and 168 hr after HSD infusion (data not shown).

In both HSD and hem+HSD groups, prothrombin (PT) and activated thromboplastin (APTT) time tended to decrease after HSD infusion compared with baseline values, but the differences were not statistically significant. (Fig. 2). At no time were PT and APTT prolonged following HSD infusion. Again, since the 48, 72 and 168 hr data were not different from baseline or the 24 hr values, they were not included in the figure.

Erythrocyte sedimentation rates (ESR) were not significantly affected by hemorrhage, but in both the HSD and hem+HSD groups, ESR increased approximately 50% over baseline values at 24 hr or 48 hr after HSD infusion (Table III). In the HSD group, ESR returned

to baseline rates at 72 hr, whereas it was still higher than baseline in the hem+HSD group. It returned to baseline rates in this group by 7 days after HSD infusion (data not shown).

In addition, platelet aggregation was not adversely affected in either group at the times measured (Fig. 3). If anything, platelet aggregation seemed to increase slightly after HSD infusion, although the differences were not significantly different from baseline. In euvolemic pigs, HSD did not prolong bleeding times at 1 or 2 hr after infusion (Table IV).

DISCUSSION

The dextran concentrations observed in the present study are similar to those previously observed in pigs and rabbits after infusion of 4 ml/kg HSD [19,24]. The 20% to 30% higher dextran concentrations in hemorrhaged than euvolemic pigs are also consistent with previous observations [19,24]. Despite these differences in concentration, the early half-life of dextran in serum was not significantly affected by hemorrhage, suggesting that renal clearance, the major component defining the early half-life of dextran [25], was not impaired in these animals. This is also consistent with our previous observations in rabbits [24].

As expected, HSD infusion raised the sodium and chloride concentrations in serum. Although serum sodium increased about 10 mEq/L in the HSD-infused animals, the sodium levels were transient and were not associated with any overt behavioral changes in these animals. The concentrations of these electrolytes increased only slightly and essentially returned to normal by 24 hr. In the present study, pigs had free access to water after the initial 4 hr experimental period. Therefore, it is not surprising that their serum electrolytes were normal at 24 hr.

The administration of dextrans to over 200,000 patients in the 1940s and 1950s led to the recognition that dextrans could induce subtle changes in hemostasis [10]. Subsequently, studies in both experimental animals and humans have shown that large doses of

high MW dextrans can prolong bleeding and clotting times, bind fibrinogen, interfere with fibrin cross-linking, reduce the plasma concentrations of factors V, VII and VIII, and inhibit platelet aggregation and platelet factor 3 activity [9,10,26]. Although these observations have led to the clinical use of low molecular weight dextrans, e.g., Dextran-40, as antithrombotics, they also raised concern that the use of dextrans as plasma volume expanders to treat hemorrhagic hypotension could exacerbate the condition they were used to treat.

In the present study, the clinical screening tests of hemostasis, PT, APTT, and platelet aggregation were determined after the IV infusion of 4 ml/kg HSD to both euvolemic and hemorrhaged pigs. PT and APTT monitor the extrinsic and intrinsic pathways of coagulation. Although each system can operate independently, they interact through factor X to regulate hemostasis. Evaluating both systems allows a more complete evaluation of the overall coagulation process. In fact, it has been suggested that the interaction between the two systems accounts for the maintenance of the clotting mechanism despite a possible impairment of the thrombin formation by dextran [27,28].

The results from the present study indicate that PT and APTT were not significantly affected following the infusion of a single 4 ml/kg dose of HSD in both euvolemic and hemorrhaged pigs. Although previous studies by us [15] and others [13] indicated that high concentrations of hypertonic saline can prolong PT, this does not appear to be the case following IV infusion of 4 ml/kg HSD. As indicated, serum sodium concentrations rise immediately after infusion and then slowly drop toward pre-infusion levels, consistent with the observation that sodium is distributed quickly throughout body compartments [15]. The present data are also consistent with our previous observations in rabbits [29] and the lack of hemostatic abnormalities noted in the human clinical trials following administration of 250 ml of HSD [8] as well as in other recent clinical studies with dextran [30].

Erythrocyte sedimentation rate (ESR) was also measured to determine the aggregating tendency of blood after HSD infusion.

Previous studies done in vitro reported that incubating human or dog blood with Dextran-70 increased ESR and therefore, erythrocyte aggregation [31,32]; a phenomenon related to higher viscosity of blood induced by Dextran-70 [33]. It is known, however, that ESR can be significantly influenced by hematocrit (Hct). In the use of HSD as a plasma volume expander for the treatment of hemorrhagic hypotension, significant decreases in Hct occur [5]. In the present study, ESR was corrected for changes in Hct since Hct was more significantly affected in the hem+HSD than the HSD group. It was observed that ESR began to slightly rise after HSD infusion and peaked at levels about 50% higher than baseline. ESR then decreased toward baseline levels. The present data are consistent with previously reported observations that infusion of Macrodex slightly increased plasma viscosity and erythrocyte aggregation [34], and further suggest that HSD infusion will not inhibit blood cell aggregation.

In addition, antithrombotic properties of dextrans are well established, yet may not be associated with any abnormalities in coagulation or bleeding time [27,35]. In our previous studies we found that platelet aggregation was decreased following incubation of a high dose of HSD with human plasma [15]. Buchanan [36] reported that adverse effects of dextran on platelet aggregation would not be anticipated until doses exceeded 1g/kg/day. Therefore, it is not surprising that platelet aggregation was not affected in the present study.

In conclusion, the present data, taken together with the available human data, indicate that the efficacy of HSD for the prehospital treatment of hemorrhagic hypotension will not be compromised by problems of impaired hemostasis.

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Table I

Effects of HSD Infusion on Mean Arteral Pressure and Heart Rate in Euvolemic and Hemorrhaged Pigs[1]

	Heart Rate (Beats/min)		Mean Arterial Pressure (mmHg)	
Time (hr)	HSD	Hem + HSD	HSD	Hem + Hem +HSD
Baseline	117±5	107±2	100±4	112±4
Post-Hemorrhage		108±10		65±6*
0.5	109±6	113±8	101±4	96±5
1	114±5	116±7	94±3	94±4
2	121±5	120±11	99±6	104±6
3	115±4	110±7	101±8	96±6
4	115±5	118±9	97±3	100±4

[1]Data expressed as mean ±S.E. For HSD group, n=11; For Hem + HSD group, n=9.
* $p<0.05$ from corresponding baseline value.

Table II

Serum Electrolytes Concertrations in Euvolemic and Hemorrhaged Pigs Infused with HSD[1]

	Sodium(mEq/L)		Potassium(mEq/L)		Chloride(mEq/L)	
Time (hr)	HSD	Hem + HSD	HSD	Hem + HSD	HSD	Hem + HSD
Baseline	150±3	147±3	4.1±0.1	4.3±0.2	103±3	100±2
Post-Hemorrhage		147±3		3.9±0.1		103±4
0.5	160±2*	154±2	4.0±0.2	3.6±0.1	110±4	111±2
1	160±3	154±2	4.1±0.1	3.7±0.2	109±4	109±5
2	161±3	156±2	4.0±0.1	4.0±0.2	110±4	109±4
3	159±3	153±2	3.8±0.1	4.0±0.1	107±3	108±5
4	160±2*	154±2	3.7±0.1	4.1±0.3	110±3	111±5
24	155±1	146±1	3.6±0.2	3.6±0.1	103±3	103±3

[1]Data expressed as mean ±S.E. For HSD group n=11 and for Hem + HSD group n=9.
*$p<0.05$ from corresponding baseline value.

Table III

Bleeding times of Euvolemic Pigs Infused with HSD[1]

Time	Bleeding time (min)
Baseline	
3.8±0.7	(4)
1 hr	
3.4±0.7	(4)
2 hr	
4.2±1.8	(2)

[1]Data expressed as mean ±S.E. (n).

Table IV

Bleeding times of Euvolemic Pigs Infused with HSD[1]

Time	Bleeding time (min)	
Baseline	3.8±0.7	(4)
1 hr	3.4±0.7	(4)
2 hr	3.9±1.1	(3)

[1]Data expressed as mean ±S.E. (n).

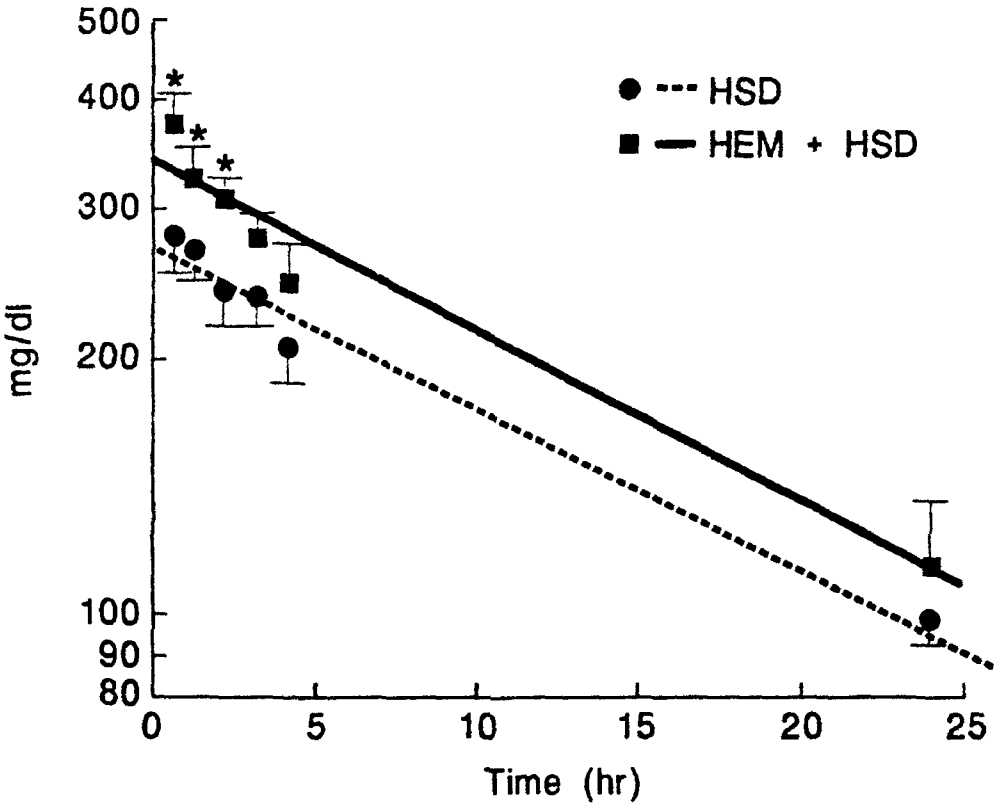

Figure 1. Serum dextran concentrations following infusion of HSD in euvolemic (n=11) and hemorrhaged (n=9) pigs. Data expressed as mean ±S.E. Concentrations in hemorrhaged animals significantly higher ($P<0.05$) than in control animals.

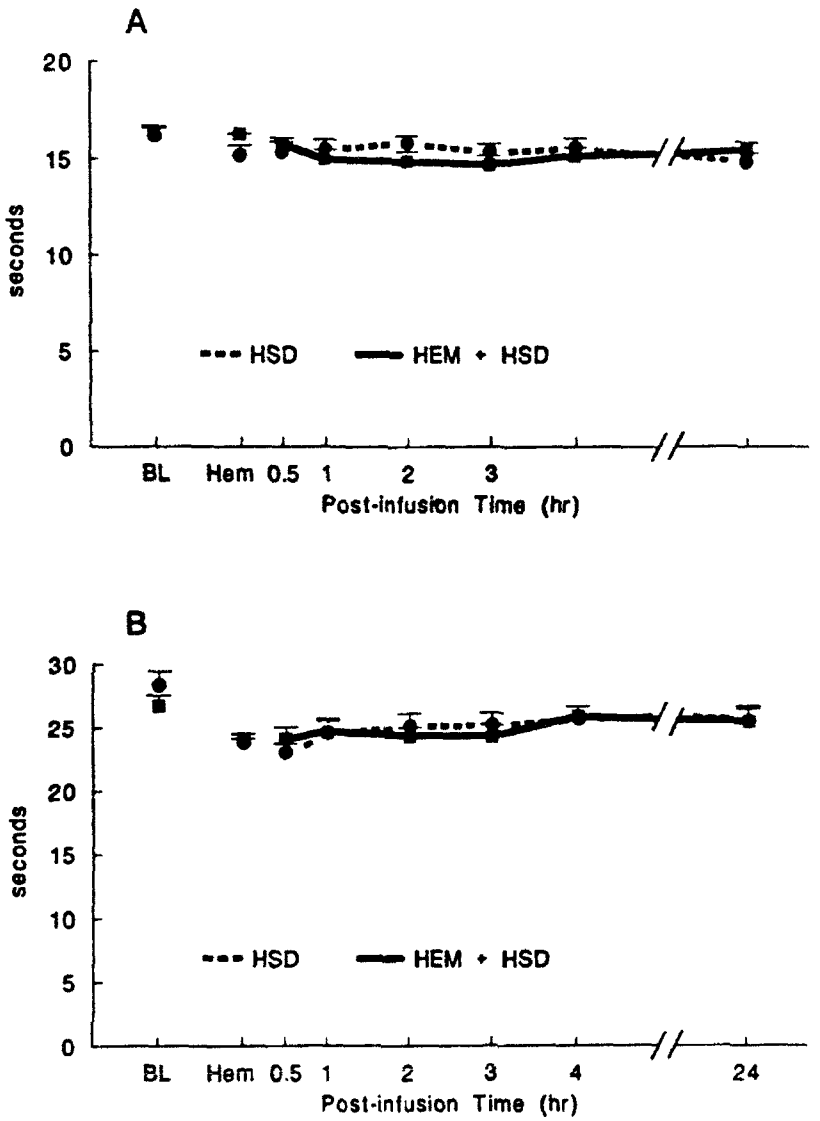

Figure 2. Prothrombin Time (A) and Activated Partial Thromboplastin Time (B) in euvolemic (n=11) and hemorrhaged (n=9) pigs infused with HSD.

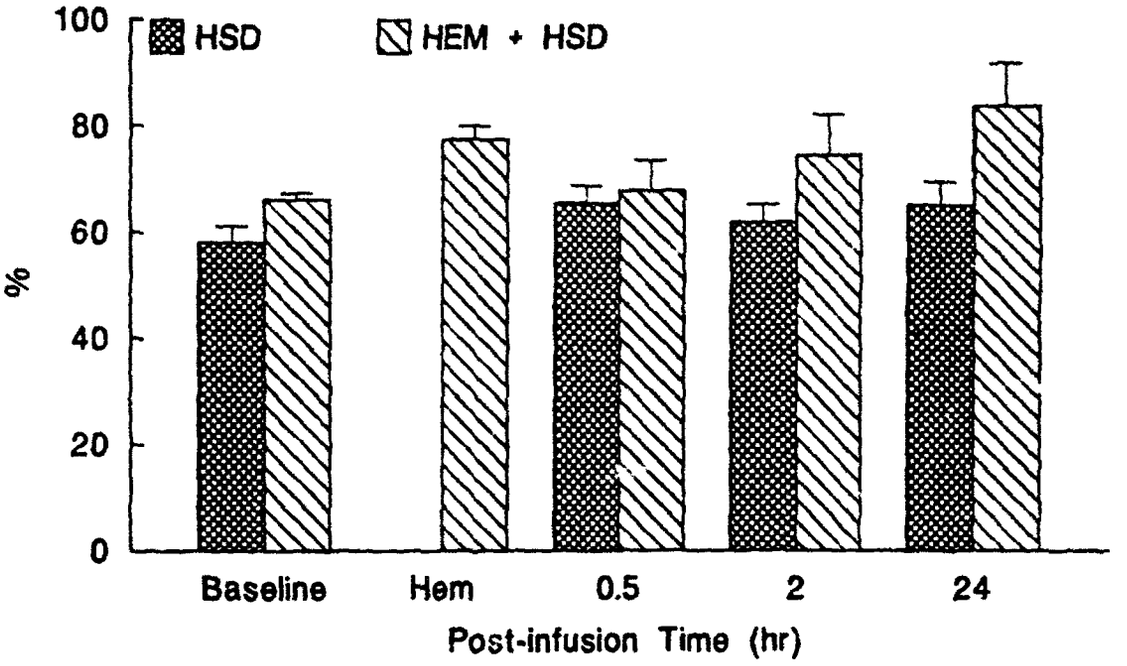

Figure 3. Platelet aggregation using ADP as agonist in euvolemic (n=5) and hemorrhaged (n=5) pigs infused with HSD.

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